Project title: TEMPO-ER — Temporal & Spatial Regulation of ER in Health and Disease

Project Summary:

State of the art: The oestrogen receptor (ERα) is the central driver of ER-positive breast cancer and the focus of a multibillion-dollar therapeutics market. Despite this centrality, fundamental aspects of ER biology in normal human breast remain unresolved. **A key question is whether ER undergoes hormone-dependent nuclear import in healthy epithelium, as textbooks propose, or whether that model reflects cancer-centric biology.** In tumours, ER is typically constitutively nuclear. Comprehensive, tissue-context analyses at subcellular resolution comparing normal vs cancer are largely absent; likewise, ER binding maps are dominated by cancer cell lines and tumours, with intact normal human breast seldom profiled.

Late appearance of ESR1 mutations suggests early non-genetic mechanisms. Clinically, ESR1 mutations are enriched in advanced and therapy-exposed disease, whereas early lesions rarely harbour such mutations. This temporal pattern implies that non-genetic changes—altered subcellular localisation, nuclear transport, chromatin binding, and cofactor usage—may prime oncogenic programmes before mutation acquisition.

Current mapping is fragmented and cancer-biased. In normal breast, conventional IHC/IF establishes that ERα is nuclear in a minority of luminal epithelial cells, but these studies stop at cellular resolution and do not resolve subcellular organisation and compartments (e.g., nuclear speckles/lamina proximity, endosomes/vesicles and lysosomes) or the nuclear-transport machinery in situ. High-resolution work that does characterise ER intranuclear dynamics (e.g., ligand-dependent redistribution, coactivator recruitment, nuclear-matrix association) has been performed predominantly in cell lines, not normal tissue. Methods capable of subcellular mapping in FFPE (e.g., multiplexed imaging, PLA) have largely been applied to tumours rather than to healthy epithelium at scale.

Gap and opportunity. To our knowledge, there is no comprehensive, tissue-context, subcellular-resolution map of ER localisation—linked to nuclear import/export proteins—and no paired comparison to ER chromatin engagement that spans normal to cancer in human breast. Ageing, particularly the peri-menopausal transition, overlays endocrine shifts and clonal expansion of ER^+ lineages, but how these factors rewire ER nuclear transport and genome engagement in situ is unknown. Addressing these gaps is essential for mechanistic understanding and for designing earlier, mechanism-grounded interventions.

Enabling technology: CosMx™ Spatial Molecular Imager provides the FFPE-compatible, subcellular, highly multiplexed readout needed to deliver—to our

knowledge, among the first comprehensive, tissue-context maps—of ER localisation linked to its nuclear-transport milieu in normal versus cancer.

Objectives: We will build a high-resolution, tissue-context framework that links ERα subcellular localisation, nuclear transport and chromatin binding across normal and malignant breast, and define how these axes shift with ageing—particularly during the peri-menopausal transition. Aim 1 will establish whether hormone-dependent nuclear import operates in healthy epithelium by quantifying ER nuclear–cytoplasmic distribution and intranuclear organisation and by situating these readouts alongside the nuclear-transport machinery in FFPE tissue. Aim 2 will determine where ER binds the genome and which protein complexes accompany it by comparing normal-like and ER-positive cancer cell lines grown as 3D spheroids, profiling ER occupancy and enhancer activity alongside quantitative proteomics, then projecting those signatures back onto tissue. Aim 3 will test how age and endocrine changes remodel localisation, transport and binding, and whether these shifts anticipate clonal expansion and early transformation.

Approaches: Tissue sources and clinical data: Archival FFPE breast tissues spanning normal, benign proliferative lesions (including atypia), DCIS, and ER+ invasive cancers; risk-reduction mammoplasties and normal-adjacent tissue; prospective surplus diagnostic material where feasible, with curated clinicopathological metadata (age, menopausal status, parity, BMI, HRT) (Breast Cancer Tissue Bank, RHM tissue bank and studies and Magnani's clinical collaborators). Cohort size and case mix will be finalised after governance and feasibility assessments. To manage pre-analytic variability, we will use harmonised SOPs, a reference TMA for run-to-run calibration, embedded positive/negative controls, and batch-effect modelling.

Aim 1 — ER subcellular localisation & nuclear transport (FFPE, subcellular resolution)

We will use CosMx™ Spatial Molecular Imager on FFPE sections to obtain subcellular, single-molecule maps of ER and its context across normal, benign, in situ and invasive specimens. Panels will capture ER and transcriptional-engagement markers, nuclear-envelope and pore components (lamins, emerin, SUN proteins; nucleoporins), the Ran GTPase cycle, importins and exportins, chaperone systems, cytoskeletal motors, and the endosome–lysosome axis (early/late endosomal markers, ESCRT, lysosomal membrane proteins, TFEB pathway, autophagy flux). We will prioritise a Tier-1 core panel for feasibility and throughput—ERα, LMNA/C, NUP153, NUP62, RAN, RCC1, KPNA2, KPNB1, XPO1, SON/SC35, LAMP1/2, EEA1, RAB7 and MKI67—expanding to Tier-2 markers only after quality control is met. From these data we will compute per-cell nuclear/cytoplasmic ratios, intranuclear texture (diffuse vs speckled/focal), distances to nuclear speckles and lamina, and neighbourhood relationships with transport proteins and organelles. Proximity ligation in selected cases will verify ER associations with importins or cofactors; PLA will be performed on serial sections co-registered to CosMx regions to respect assay requirements. We will derive a pre-specified Transport Score that integrates ER nuclear/cytoplasmic ratio,

proximity to nucleoporins, abundance of importins/exportins and RNAPII pSer2/5 engagement. Segmentation and distance metrics will be benchmarked using cell-mix controls and synthetic phantoms, with orthogonal validation by multiplex IF (or MIBI-TOF) on serial sections and uncertainty reported via bootstrapped confidence intervals. The outcome will be a tissue-context Subcellular ER, Transport & Endo-lysosomal Atlas that contrasts physiological and cancer-specific states.

Aim 2 — ER chromatin binding & immediate programmes (3D models; proteomics)

To interrogate ER chromatin engagement and complexes without FFPE constraints, we will culture normal-like and ER-positive breast cell lines as matrix-embedded 3D spheroids and expose them to physiological oestrogen, withdrawal, and SERMs/SERDs. We will profile ER occupancy (ChIP-seq or CUT&RUN on fresh/frozen material) together with enhancer activity (ATAC-seq), and in parallel deploy quantitative mass spectrometry to define ER co-regulator assemblies, chromatin-associated proteomes and phosphorylation states within transport and cofactor networks under the same conditions. Model-derived signatures will then be projected onto tissue using shared genes and proteins, enabling a direct normal–cancer comparison of ER binding modules and cofactor use.

To strengthen functional interpretation, proteomics will include TMT-labelled nuclear/chromatin fractionation, targeted ER IP–MS (±E2, ±SERD), and phospho-proteomics focused on transport/cofactor pathways. We will pre-register sentinel complexes (ER–SRC-1/p300, ER–HSP90, ER–KPNA2/KPNB1) whose assembly/disassembly will be quantified. Scope is controlled by designating core assays (ER ChIP-seq/CUT&RUN + H3K27ac/ATAC-seq; proteomics in 2–3 lines across ±E2 and one anti-oestrogen) with optional extensions only if timelines allow.

Aim 3 — Ageing, peri-menopause, and early transformation

We will stratify tissues by age and menopausal status—with emphasis on peri-menopause—and apply Aim-1 and Aim-2 readouts across strata to characterise ERα trafficking in situ. Analyses will focus on ER nuclear–cytoplasmic balance, intranuclear organisation, proximity to nucleoporins, abundance of importins/exportins, and coupling to lysosomal/endosomal markers and RNAPII engagement. Where feasible, short-term perturbations in fresh material or 3D models will track ER localisation dynamics using nuclear import/export reporters under physiological oestrogen pulses, withdrawal and SERM/SERD conditions, thereby linking endocrine context to transport behaviour.

Mixed-effects modelling will integrate localisation, transport and binding features into an ER Early Transformation Signature (ETS) and a quantitative ER Trafficking & Engagement Score (ETES), which we will test in independent human cohorts. To better control endocrine transitions, we will complement human tissues with an ACI rat model of hormone-driven mammary biology (peri-menopause–relevant states).

This component will be material-only for the PhD student: tissues and metadata will be provided by experienced colleagues in the lab; the student will perform CosMx readouts and targeted validations on supplied sections. This optional extension mitigates human cohort variability while avoiding overextension into in-vivo work.

Feasibility, training, and milestones: The PhD student will lead CosMx spatial profiling, digital pathology, laser microdissection, 3D spheroid culture, fresh/frozen ChIP-seq/CUT&RUN, quantitative proteomics and integrative analysis (R/Python). Year 1 will prioritise governance, cohort assembly and CosMx panel optimisation, culminating in Atlas v0 (pilot 10–15 cases) and a pre-registered analysis plan. A decision gate after Atlas v0 will lock the Tier-1 panel, QC thresholds and power assumptions. Year 2 will deliver chromatin and proteomic maps from 3D models, expand to Atlas v1 with scaled tissue cohorts, and submit a comparative normal–cancer localisation/transport manuscript. Year 3 will perform the ageing analyses (including ACI rat material if invoked), derive and test the ETS, and release datasets, SOPs and tools. Proteomics and advanced modelling are staged with core-facility support to keep workload tractable.

Laboratory environment, supervision & resources: Supervision is provided primarily by Luca Magnani (ICR), with co-supervision from Dalia Rosano (Staff Scientist) and Adrian Tebar (Postdoc), and clinical–translational mentoring from Nick Turner (Oncology). The student is embedded in a multidisciplinary lab spanning receptor biology, chromatin and spatial methods, quantitative analysis and clinical pathology. Core infrastructure includes CosMx SMI, high-content microscopy, laser microdissection, proteomics mass spectrometry, sequencing platforms and institutional HPC. Access to curated FFPE cohorts and collaborator biobanks—finalised post-governance—together with public datasets ensures both discovery and validation. Dedicated consumables, core-facility budgets, training and conference support underpin delivery.

Impact: This programme will deliver a mechanistic account of how ER enters and resides in the nucleus and where it binds in normal human breast versus cancer, reveal how peri-menopausal ageing reconfigures these axes, and provide openly available atlases, cistrome maps and analysis pipelines that inform basic research and next-generation ER therapeutics. The ETS derived here offers a path toward earlier, biology-anchored biomarkers while keeping the central deliverable firmly on understanding ER biology.

Supervisory Team: Prof. Luca Magnani and Prof. Nick Turner

Clinical Specialities: Pathology/Oncology